Tryptase-catalyzed core histone truncation: A novel epigenetic regulatory mechanism in mast cells

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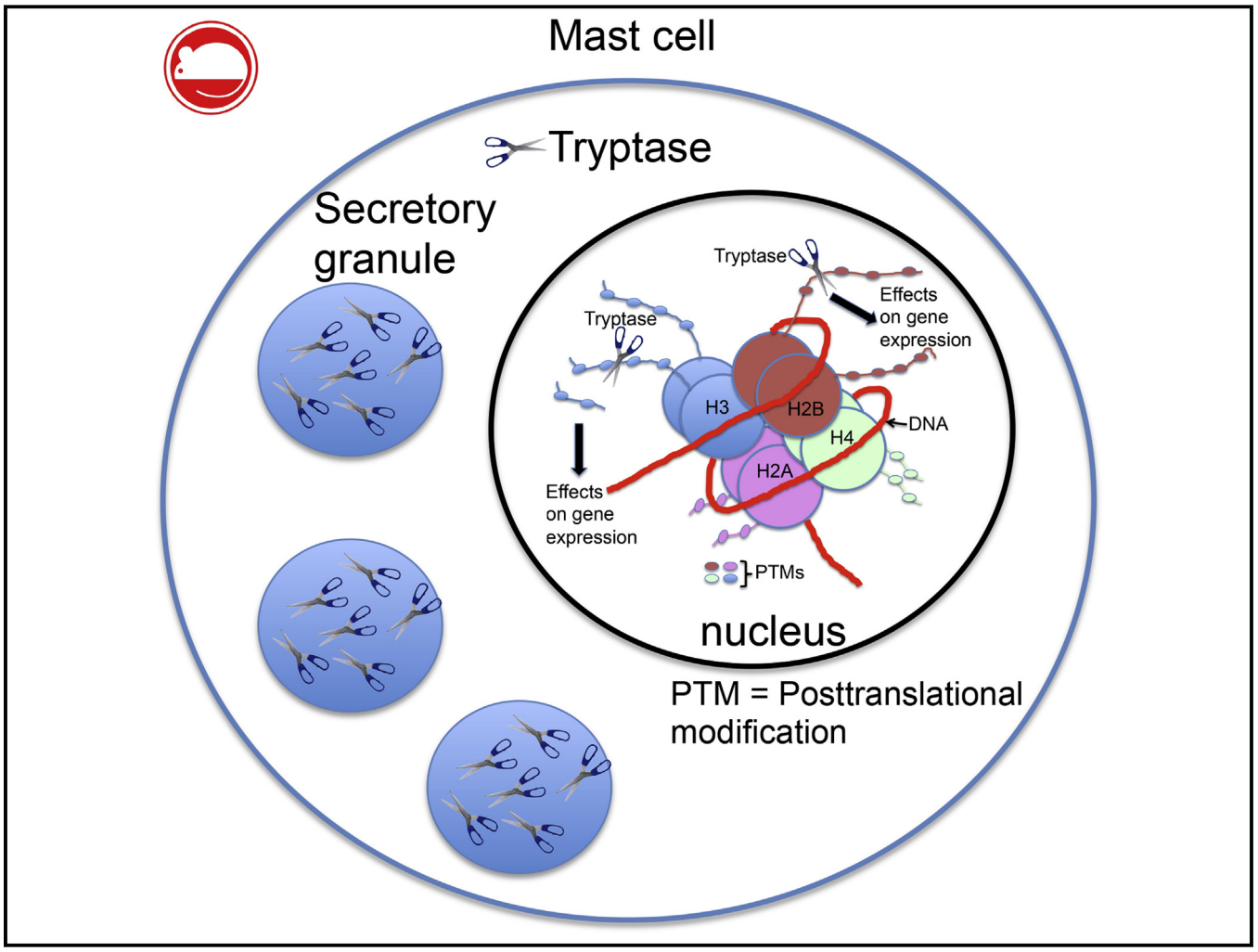
GRAPHICAL ABSTRACT



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Background: Mast cells are key effector cells in allergic reactions. When activated to degranulate, they release a plethora of bioactive compounds from their secretory granules, including mast cell-restricted proteases such as tryptase. In a previous study, we showed that tryptase, in addition to its intragranular location, can be found within the nuclei of mast cells where it truncates core histones at their N-terminal ends.
Objective: Considering that the N-terminal portions of the core histones constitute sites for posttranslational modifications of major epigenetic impact, we evaluated whether histone truncation by tryptase could have an impact on epigenetic events in mast cells.
Methods: Mast cells were cultured from wild-type and tryptase null mice, followed by an assessment of their profile of epigenetic histone modifications and their phenotypic characteristics.
Results: We show that tryptase truncates nucleosomal histone 3 and histone 2B (H2B) and that its absence results in accumulation of the epigenetic mark, lysine 5-acetylated H2B. Intriguingly, the accumulation of lysine 5-acetylated H2B was cell age–dependent and was associated with a profound upregulation of markers of non–mast cell lineages, loss of proliferative control, chromatin remodeling as well as extensive morphological alterations.
Conclusions: These findings introduce tryptase-catalyzed histone clipping as a novel epigenetic regulatory mechanism, which in the mast cell context may be crucial for maintaining cellular identity. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *Mast cells, epigenetics, core histones, histone acetylation, tryptase, mMCP6, secretory granules, serglycin, serglycin proteoglycan, H2B, H2BK5ac*

Mast cells (MCs) are hematopoietic cells with a wide impact on various pathophysiological settings, most notably allergy but also cancer, atherosclerosis, obesity, and autoimmune disease.[1,2] A hallmark feature of MCs is their high content of secretory granules, filled with a plethora of bioactive substances such as bioactive amines (histamine and serotonin), cytokines, growth factors, proteoglycans of serglycin type and multiple proteases including tryptases, chymases, and carboxypeptidase A3 (CPA3).[3,4] Although the general notion is that the default fate of these granule-contained compounds is to be delivered to the extracellular milieu where they influence various inflammatory processes, we recently showed that one of the MC granule-contained proteases, tryptase, can also be found in the nucleus.[5] Moreover, we showed that nuclear tryptase has the ability to proteolytically remove core histone N-terminal tails, thereby acting as a histone "clippase." It is now established that the N-terminal tails of core histones constitute sites for posttranslational modification (PTM) events that have a wide epigenetic impact.[6-8] Conceivably, tryptase-catalyzed histone clipping may thus cause changes in the pattern of epigenetic histone modifications, potentially affecting gene expression and cellular phenotype. Here we investigated this possibility and demonstrate that the absence of tryptase causes an age-dependent accumulation of 1 epigenetic mark–acetylated histone 2B lysine 5 (H2BK5ac). Furthermore, extensive effects on cellular phenotype accompany this change to the chromatin landscape. Thereby, tryptase-catalyzed N-terminal clipping of H2B emerges as a novel epigenetic regulatory mechanism in MCs.

Abbreviations used

CFSE:	Carboxyfluorescein diacetate succinimidyl ester
ChIP-seq:	Chromatin immunoprecipitation-sequencing
CPA3:	Carboxypeptidase A3
H:	Histone
H2BK5ac:	Acetylated histone 2B lysine 5
H3K9me3:	H3 trimethylated at lysine 9
MC:	Mast cell
mMCP:	Mouse mast cell protease
PTM:	Posttranslational modification
qPCR:	Quantitative real-time RT-PCR
TEM:	Transmission electron microscopy
WT:	Wild type

METHODS

Animals and cells

Wild-type (WT), serglycin$^{-/-}$,[9] and mouse mast cell protease (mMCP)-6$^{-/-}$[10] mice were on C57BL/6J genetic background. Bone marrow-derived MCs were cultured in IL-3–containing medium as described.[11]

Methods

Proliferation was assessed by carboxyfluorescein diacetate succinimidyl ester (CFSE) dilution. Cell morphology was assessed by transmission electron microscopy (TEM)[12] or light microscopy after staining with May Grünwald/Giemsa or toluidine blue.[11] Western blot analysis,[11] chromatin immunoprecipitation-sequencing (ChIP-seq) analysis,[13] oligonuclesome purification,[14] and quantitative real-time RT-PCR (qPCR) analysis[11] were carried out as described. Immunohistochemical staining and confocal microscopy was performed using antibodies to H2BK5ac, mMCP6, and CPA3.

For further details, see the Methods section in this article's Online Repository at www.jacionline.org.

RESULTS

Absence of tryptase or serglycin in MCs results in age-dependent loss of proliferative control

We showed previously that MC tryptase (mMCP6) has the ability to proteolytically remove the N-terminal tails of core histones.[5] To assess whether this can affect the epigenome of the MCs such that their phenotype is altered, we developed bone marrow-derived MCs from WT and mMCP6$^{-/-}$ mice[15] and assessed their characteristics. In addition, we developed MCs from mice lacking serglycin, a proteoglycan that is crucial for the storage of mMCP6 in MCs.[9] Hence, the phenotypic characteristics of tryptase-deficient cells should be mimicked in cells lacking serglycin.

Approximately equal numbers of bone marrow cells were obtained from WT, serglycin$^{-/-}$ and mMCP6$^{-/-}$ mice and addition of MC-driving growth factors (IL-3–containing medium) to the bone marrow cells produced an expansion of the cultures at approximately equal rates up to ~3 months (Fig 1, *A, B*, and *D*). However, when cultures were followed over an extended time, we noticed that cultures of mMCP6$^{-/-}$ cells expanded at a considerably higher rate than did cultures of WT cells (Fig 1, *B* and *D*). Notably, the onset of exaggerated expansion rates differed between individual cultures, with some cultures showing an onset at ~3 months, whereas a delayed onset was seen in others (Fig 1, *D*). Expansion of the MC niche was also seen *in vivo* when

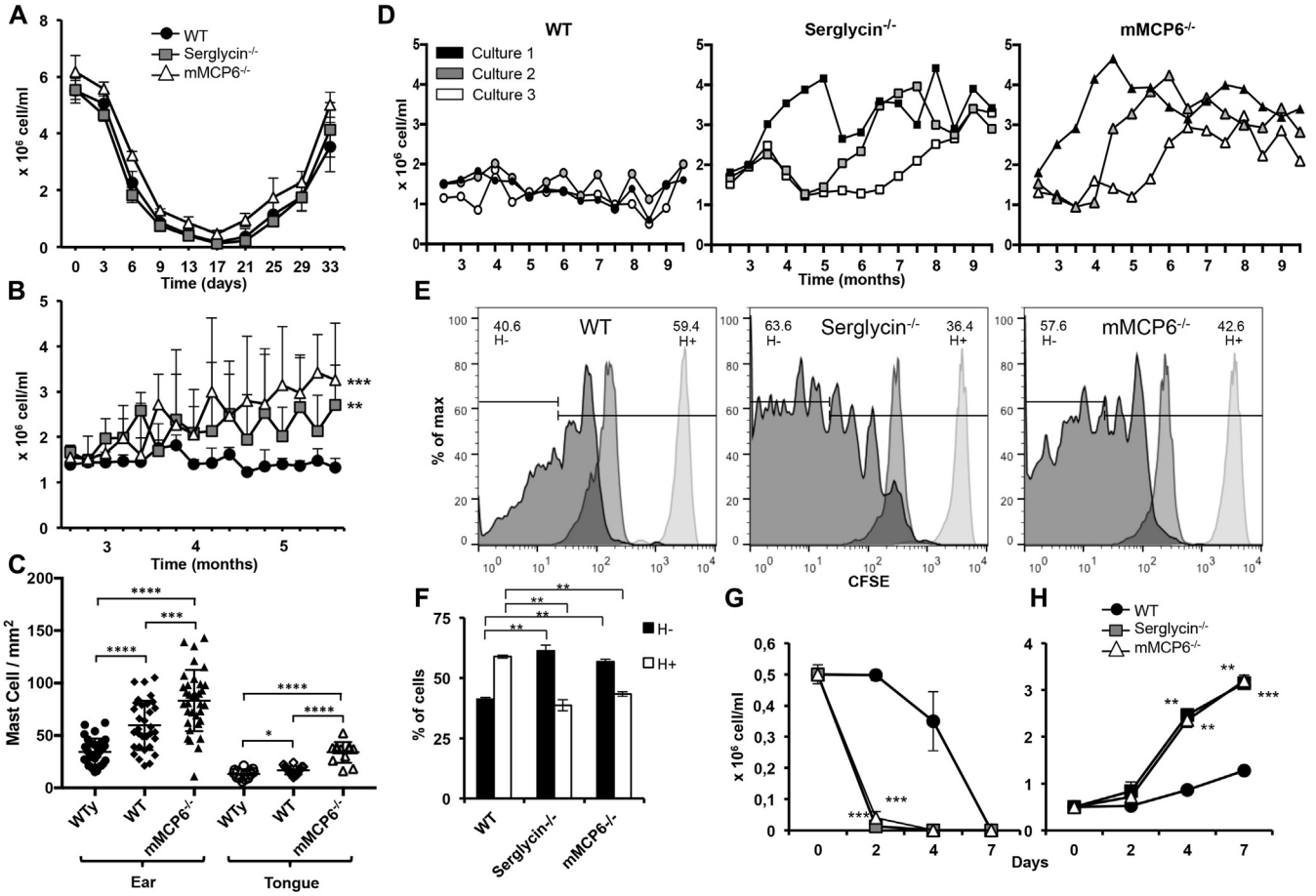


FIG 1. Absence of tryptase or serglycin results in age-dependent loss of proliferative control. **A**, Bone marrow cells from WT, serglycin$^{-/-}$, or mMCP6$^{-/-}$ mice were differentiated into MCs and were enumerated at the indicated time points. **B**, MCs were cultured for extended time periods (up to 6 months) and cells were enumerated before passages (n = 3). **C**, MCs were enumerated in the ear skin and tongue of young (4 months) and old (2 years) mice, using toluidine blue staining (n = 30-34 for ear skin sections; n = 12-16 for tongue sections). **D**, Cell counts of individual MC cultures kept for up to 9.5 months. **E**, CFSE dilution experiment performed on 5-month-old MCs. Cells were analyzed at day 0 (*light gray*), day 5 (intermediated *gray* shade), and day 10 (*dark gray*) after CFSE labeling. **F**, Quantification of CSFE labeling data, using the gates (H−, H+) specified in (*E*) (n = 3). **G**, Five-month-old MCs of the indicated genotypes were deprived of growth factors, followed by assessment of residual viable cells (trypan blue staining) at the time points indicated. **H**, Parallel control experiment to (*G*) confirming more rapid proliferation of serglycin$^{-/-}$ and mMCP6$^{-/-}$ cells than WT cells in the presence of IL-3. Data are presented as mean values ± SEM; **P* < .05, ***P* < .01, ****P* < .001, and *****P* < .0001. *WTy*, WT (young).

analyzing tissues from aging mMCP6$^{-/-}$ mice (Fig 1, *C*; see Fig E1 in this article's Online Repository at www.jacionline.org). Similar to mMCP6$^{-/-}$ cells, serglycin$^{-/-}$ cultures exhibited age-dependent increased expansion rates (Fig 1, *B* and *D*). To evaluate whether the increased cell number expansion seen in mMCP6$^{-/-}$ and serglycin$^{-/-}$ cultures was a result of increased proliferation, we performed a CFSE dilution experiment. Indeed, aged mMCP6$^{-/-}$ and serglycin$^{-/-}$ cells showed a markedly higher rate of proliferation than did corresponding WT cells (Fig 1, *E* and *F*). In contrast, similar rates of proliferation were seen in WT, mMCP6$^{-/-}$, and serglycin$^{-/-}$ cells taken before the observed onset of increased expansion rates (data not shown). The numbers of apoptotic cells did not differ among WT, mMCP6$^{-/-}$, and serglycin$^{-/-}$ cultures (data not shown), reinforcing that the increased expansion rates seen in mMCP6$^{-/-}$ and serglycin$^{-/-}$ cultures is a result of increased proliferation. The increased proliferation of mMCP6$^{-/-}$ and serglycin$^{-/-}$ cells was not associated with reduced growth factor dependency (Fig 1, *G* and *H*). In fact, mMCP6$^{-/-}$ and serglycin$^{-/-}$ cells underwent cell death at a considerably higher rate than WT cells after growth factor deprivation (Fig 1, *G* and *H*).

Tryptase- or serglycin-deficiency causes age-dependent morphological alterations

No major morphological differences between WT and mMCP6$^{-/-}$ cells were seen in young cultures (up to ~3 months; not shown). However, after extended culture times, we noted that mMCP6$^{-/-}$ cells exhibited a marked increase in cellular size, as assessed both by side/forward scatter analysis (Fig 2, *A-C*) and by inspection with light microscopy (Fig 2, *D-F*). An increase in cellular size was also observed in serglycin$^{-/-}$ cells (Fig 2, *A-E*). Moreover, we noted the frequent appearance of multinucleated and giant cells in cultures of

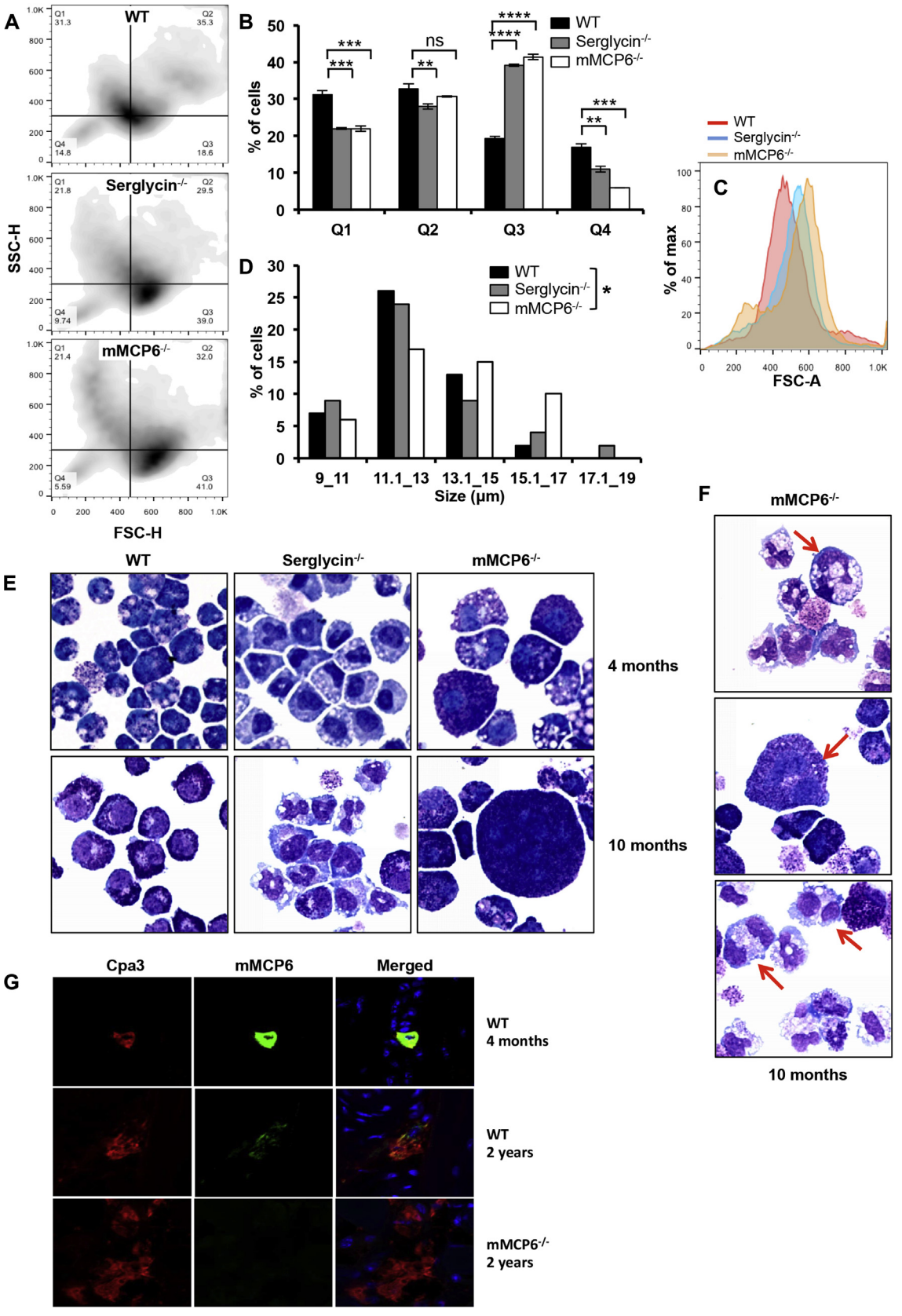
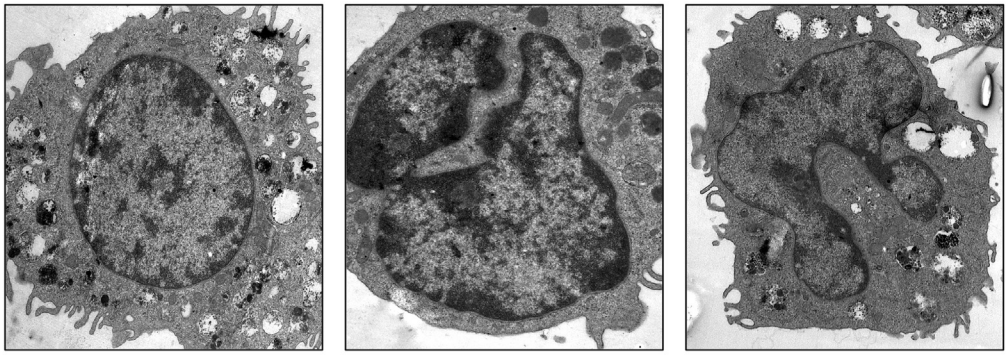


FIG 2. Tryptase- or serglycin-deficiency causes age-dependent morphological alterations. **A** and **C**, Representative dot plots (*A*) and histogram (*C*) of forward/side scatter fluorescence-activated cell sorting analysis of 5-month-old cells showing larger size (*FSC-H*) of serglycin$^{-/-}$ and mMCP6$^{-/-}$ MCs versus WT controls. **B**, Quantification of the data from the forward/side scatter analysis (n = 3). **D**, Cell size distribution as determined by cell diameter. **E**, Representative May Grünwald/Giemsa-stained cytospin slides of 4- and 10 month-old MCs. **F**, Examples of dramatic changes (*red arrows*) in nuclear architecture of aged mMCP6$^{-/-}$ MCs. **G**, Confocal microscopy analysis of mMCP6 and CPA3 (MC granule marker) expression in tongue tissue from young (4 months) and aged (2 years) WT and mMCP6$^{-/-}$ mice. Data are presented as mean values ± SEM; **P* < .05, ***P* < .01, ****P* < .001, and *****P* < .0001. *FSC-A*, Forward scatter; *ns*, not significant; *Q*, quartile; *SSC-H*, side scatter.

A



B

	WT	Serglycin$^{-/-}$	mMCP6$^{-/-}$
Round nuclei #	1.0 ± 0.06 (18)	0.32 ± 0.11 (19)	0.06 ± 0.06 (18)
		p < 0.0001 vs WT	p < 0.0001 vs WT
Nuclear lobes #	0.020 ± 0.02 (18)	0.47 ± 0.25 (19)	0.22 ± 0.15 (18)
		ns* vs WT	ns vs WT
Small contraction #	0.56 ± 0.56 (18)	0.54 ± 0.16 (19)	0.92 ± 0.17 (18)
		ns vs WT	ns vs WT
Deep contraction #	0.028 ± 0.028 (18)	0.26 ± 0.1 (19)	0.44 ± 0.20 (18)
		p < 0.05 vs WT	p < 0.05 vs WT

Values are the mean/cell section of the indicated number of cell sections ( ) ± SEM.
p is calculated according to Student's t test, unpaired and two tailed.
* ns = not significant.

FIG 3. The absence of tryptase mMCP6 or serglycin in MCs causes nuclear remodeling. **A**, TEM analysis of 5-month-old WT, serglycin$^{-/-}$, and mMCP6$^{-/-}$ MCs. Note the rounded nuclear shape of WT cells and the lobulated structure of MCs from serglycin$^{-/-}$ and mMCP6$^{-/-}$ MCs. **B**, Quantification of nuclear characteristics.

mMCP6$^{-/-}$ MCs (Fig 2, *E* and *F*), which were absent in WT cultures (Fig 2, *E*). Similar morphological changes were also seen *in vivo*, as manifested in tissue MCs of aging mMCP6$^{-/-}$ mice (Fig 2, *G*; see Fig E2 in this article's Online Repository at www.jacionline.org). It was also apparent that serglycin$^{-/-}$ and mMCP6$^{-/-}$ cells frequently showed dramatic changes in their nuclear architecture, with many cells displaying a multilobulated or kidney-shaped rather than round nucleus (Fig 2, *E* and *F*).

We also analyzed cells by TEM. As seen in Fig 3, WT cells had a rounded nucleus with defined nucleolus and possessed numerous cytoplasmic granules with mixed regions of dense core and electron translucent areas, in agreement with previous studies.[16] In contrast, the nuclei of mMCP6$^{-/-}$ and serglycin$^{-/-}$ cells showed signs of extensive chromatin remodeling, exhibiting marked deviation from the rounded nuclear shape of WT cells and with many cells showing a more macrophage-like or multilobulated morphology (Fig 3). Together, the light microscopy and TEM analysis indicate that the absence of mMCP6 or serglycin has a large, cell age–dependent impact on the chromatin organization of MCs.

Tryptase is localized to chromatin and causes clipping of nucleosomal H2B and H3

As chromatin remodeling is a hallmark manifestation of epigenetics, the data above support our hypothesis that the absence of the tryptase-serglycin axis may influence epigenetic regulatory mechanisms in MCs. Hence, we set out to investigate this possibility. In agreement with our previous findings,[5] the absence of mMCP6 resulted in a dramatic reduction in the levels of truncated histone 3 (H3) and H2B (Fig 4, *A*). In further agreement with this notion, incubation of WT MCs with a selective tryptase inhibitor (nafamostat mesylate) caused a substantial reduction in the truncation of H2B and H3 (Fig 4, *B*). Next, we assessed whether tryptase can cleave histones that are incorporated into nucleosomes, the latter is a prerequisite for having an impact on epigenetic regulation. To address this, we purified nucleosomes from HeLa cells and incubated these with recombinant tryptase. Indeed, tryptase was able to truncate histones in a nucleosomal context, with H2B and H3 being particularly susceptible (Fig 4, *C*). H2A was less susceptible and H4 was largely resistant to tryptase-catalyzed clipping (Fig 4, *C*). A dose response assay showed that tryptase was efficient at concentrations down to 0.25 ng/mL (Fig 4, *C*). Moreover, time course analysis revealed that tryptase-catalyzed clipping of nucleosomal histones was a very rapid event (Fig 4, *D*). Although we showed previously by confocal microscopy that tryptase is found in the nucleus of MCs,[5] it has not been demonstrated whether tryptase is in fact associated with chromatin. To clarify this issue, we prepared cytosolic, nuclear, and chromatin fractions and assessed the mMCP6 levels in these fractions by immunoblot analysis. As expected, the cytosolic fractions had high levels of tryptase, originating

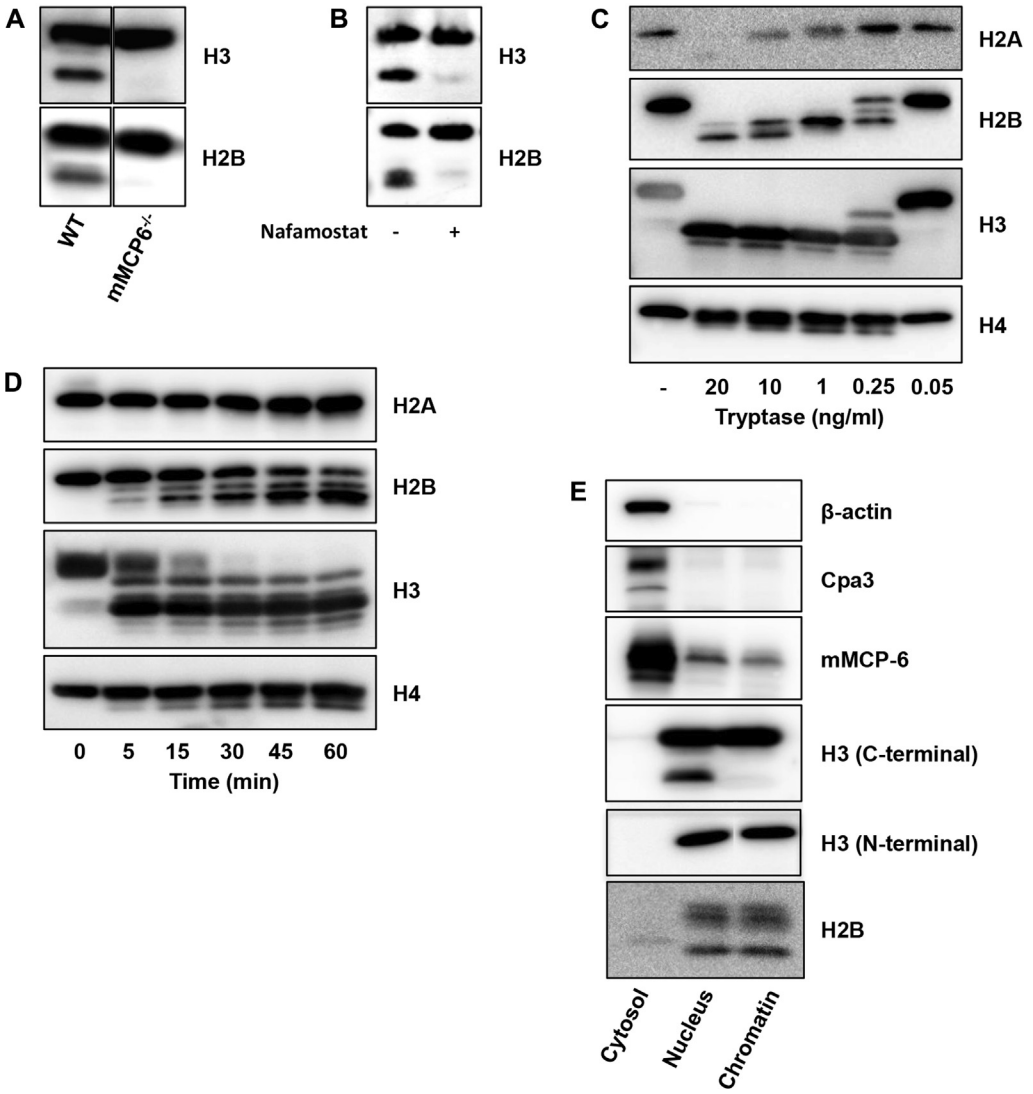


FIG 4. Tryptase is associated with chromatin and causes clipping of nucleosome-associated H2B and H3. **A**, Western blot analysis confirming that the absence of tryptase (mMCP6) results in defective clipping of H3 and H2B. **B**, WT MCs were cultured with tryptase inhibitor (nafamostat mesylate; 1 μmol/L) for 48 hours, followed by Western blot analysis for H2B and H3 processing. **C**, Oligonucleosomes prepared from HeLa cells were subjected for 30 minutes to treatment with increasing concentrations of recombinant tryptase, followed by Western blot analysis for H2A, H2B, H3, and H4. **D**, Time course analysis showing clipping of core histones after addition of 0.25 ng/mL tryptase to oligonucleosomes. **E**, Cytosol, nuclear, and chromatin fractions were extracted from MCs and were subjected to Western blot analysis for β-actin, CPA3 (MC granule marker), tryptase (mMCP6), H3 (antibody to C- and N-terminal, respectively), and H2B.

from the cytoplasmic secretory granules (Fig 4, *E*). It was also evident that mMCP6 could be found both in the nuclear and chromatin fractions. In contrast, another prominent granule protease, CPA3, was only detected in the cytosolic fraction. Hence, these data support that tryptase is associated with chromatin in MCs. It was also apparent that truncated H2B was found within the chromatin (as well as in the nuclear fraction), whereas truncated H3 was seen in the nuclear but not in the chromatin fraction (Fig 4, *E*).

Tryptase regulates H2B lysine 5 acetylation

To evaluate whether tryptase-catalyzed histone clipping influences the levels of epigenetic histone marks, we performed analysis of histone PTMs using antibodies detecting histone modifications of known major biological significance. We thereby focused on PTMs of H3 and H2B because our data above suggested that these were particularly susceptible to tryptase-catalyzed clipping (see Fig 4). As shown in Fig 5, *A* and *F*, immunoblot signals were obtained for the H3 trimethylated at lysine 9 (H3K9me3), H3K27me3, H3K9ac, H3K14ac, and H3K4me1 modifications of H3. However, signals were of approximately equal intensity in WT, serglycin$^{-/-}$, and mMCP6$^{-/-}$ cells. For H2B, signals corresponding to the H2BK5ac, H2BK9ac, and H2BK12ac modifications were detected. Intriguingly, although the signals for the H2BK9ac and H2BK12ac marks were equal among the genotypes, there was an increase in H2BK5ac in both mMCP6$^{-/-}$ and serglycin$^{-/-}$ MCs in comparison with WT controls (Fig 5, *A* and *B*), suggesting that tryptase clipping

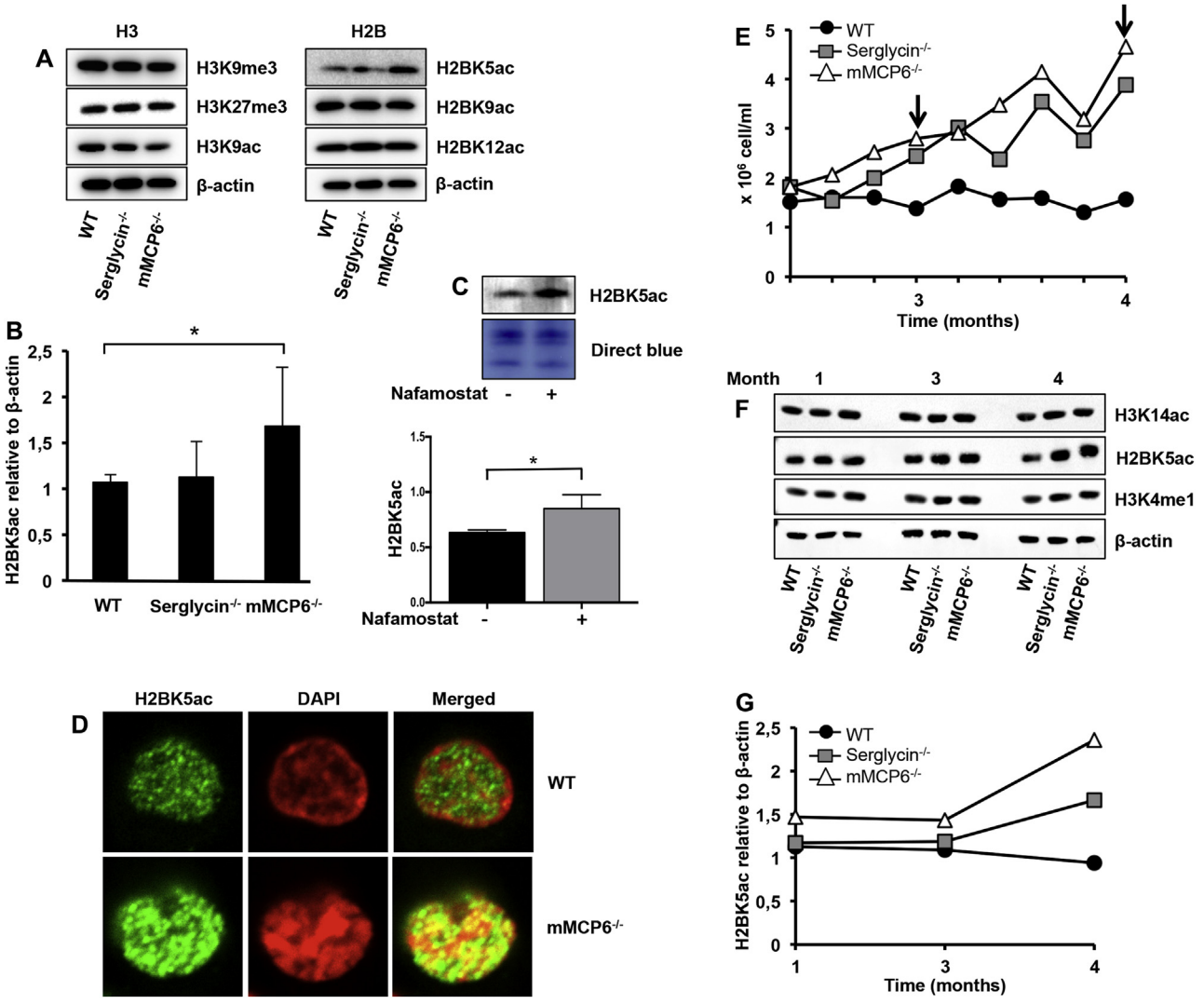


FIG 5. Tryptase regulates H2B lysine 5 acetylation. **A**, Western blot analysis showing the levels of H3 and H2B PTMs in WT, serglycin$^{-/-}$, and mMCP6$^{-/-}$ MCs (3 months old). Note the increase of H2BK5ac in mMCP6$^{-/-}$ cells. **B**, Quantification of Western blot analysis for the levels of H2BK5ac relative to the levels of β-actin (n = 6). **C**, WT MCs were incubated for 48 hours with tryptase inhibitor (nafamostat mesylate; 1 μmol/L), followed by Western blot analysis for H2BK5ac and quantification of data (n = 4). **D**, Confocal microscopy analysis verifying the upregulated expression of H2BK5ac in mMCP6$^{-/-}$ cells versus WT controls. Nuclei were visualized using 4′-6-diamidino-2-phenylindole, dihydrochloride (*DAPI*). **E**, Cell numbers in WT, serglycin$^{-/-}$, and mMCP6$^{-/-}$ cultures, with *arrows* indicating time points used for PTM analysis in (*F*). **F** and **G**, Comparison of the levels of histone PTMs in 1-, 3-, and 4-month-old cultures of WT, serglycin$^{-/-}$, and mMCP6$^{-/-}$ MCs as shown by Western blot analysis (*F*) and quantification of the H2K5ac bands relative to β-actin (*G*). Note the age-dependent increase in H2BK5ac in aging mMCP6$^{-/-}$ and serglycin$^{-/-}$ cells versus WT controls. Data are presented as mean values ± SEM; *$P < .05$.

of H2B affects this particular epigenetic modification. In agreement with this, incubation of WT MCs with tryptase inhibitor resulted in significant accumulation of the H2BK5ac mark (Fig 5, *C*). Furthermore, confocal microscopy analysis revealed a markedly more intense staining for H2BK5ac in the nuclei of mMCP6$^{-/-}$ versus WT cells (Fig 5, *D*). Accumulation of H2BK5ac was only seen in cell cultures after prolonged culture times (from ~3-4 months) (Fig 5, *E-G*). Hence, the accumulation of H2BK5ac occurs in a cell age–dependent manner in mMCP6$^{-/-}$ and serglycin$^{-/-}$ cells, and there is thus a correlation between the onset of increased cellular proliferation (see Fig 1) and accumulation of this particular PTM.

The absence of tryptase results in upregulated expression of non-MC lineage markers

Given the suggested role for H2BK5ac as a marker for active chromatin,[17] we next investigated how the epigenetic effects of tryptase deficiency affect gene expression. To address this issue, we first screened for genes differentially expressed in mMCP6$^{-/-}$ cells (taken after 4 months of culture) compared with WT cells using a microarray analysis of total RNA. Indeed, the gene array analysis indicated that numerous genes were extensively upregulated in mMCP6$^{-/-}$ cells (data not shown). Several of these represented markers of non-MC lineages, including *Emr1* (also known as F4/80 or *Adgre1*), which

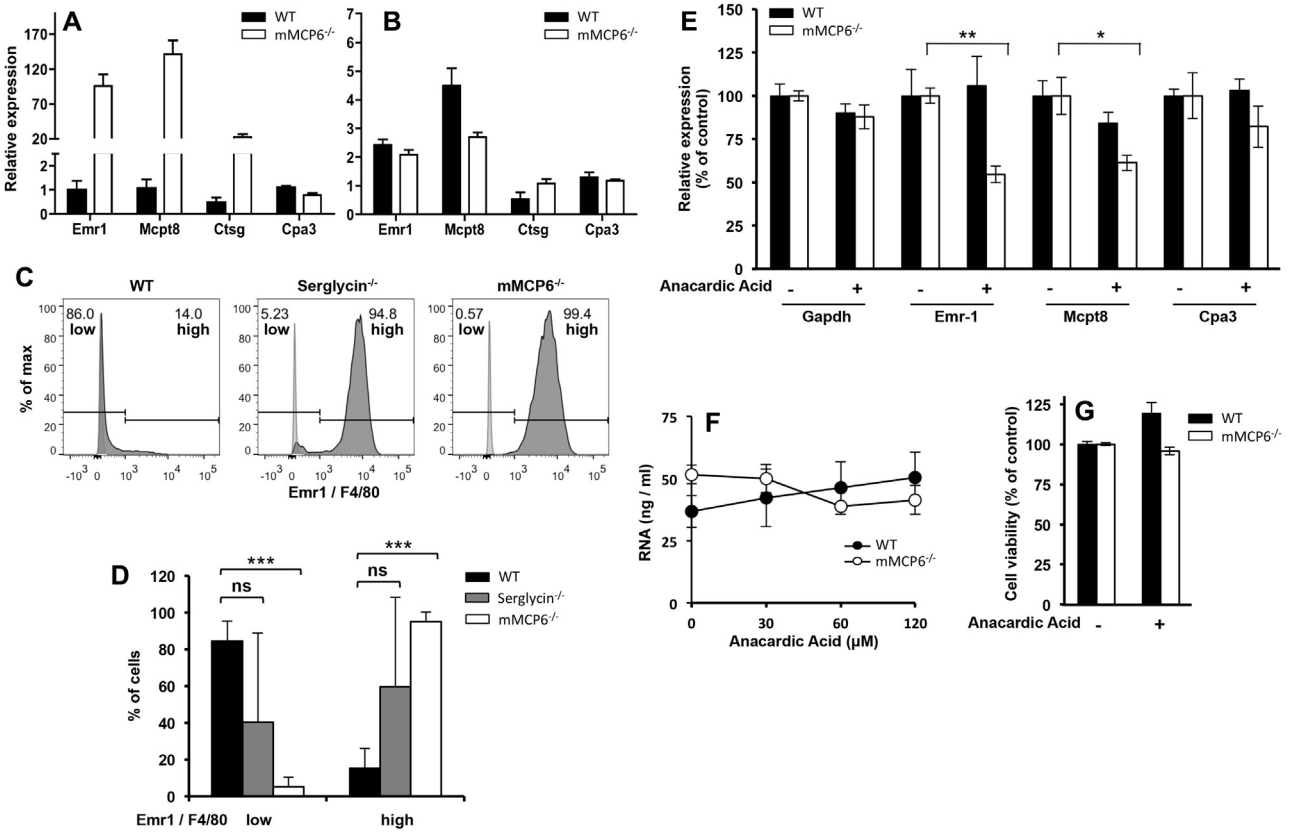


FIG 6. Upregulation of non-MC lineage markers in aging tryptase-deficient MCs. Total RNA was extracted from aging (5-month-old **[A]**) or 6-week-old **(B)** WT and mMCP6$^{-/-}$ MCs and was analyzed by qPCR for the expression of non-MC lineage genes (*Emr1, Mcpt8, Ctsg*). Results are shown as expression relative to the house-keeping gene (*Hprt*). **C**, Flow cytometry analysis verifying upregulated cell surface expression of *Emr1*/F4/80 in 5-month-old mMCP6$^{-/-}$ and serglycin$^{-/-}$ MCs versus WT controls. *Light gray-shaded* area, isotype control. **D**, Percentage of Emr1/F4/80^{high} WT, serglycin$^{-/-}$, and mMCP6$^{-/-}$ cells in 5-month-old cell cultures (n = 3). **E**, WT and mMCP6$^{-/-}$ MCs were treated with histone acetyltransferase inhibitor (anacardic acid; 60 μmol/L) for 4 hours or left untreated, followed by qPCR analysis for the expression of Gapdh, Emr1, Mcpt8, and Cpa3 as indicated. Results are shown as percentage of control (untreated samples) relative to Hprt expression. **F**, Quantification of the amount of total RNA extracted from WT and mMCP6$^{-/-}$ MCs (equal numbers of cells from each genotype) after 4-hour treatment with different concentrations of anacardic acid. **G**, Cell viability of WT and mMCP6$^{-/-}$ MCs treated for 4 hours with 60 μmol/L anacardic acid. Data are presented as mean values ± SEM; $*P < .05$, $**P < .01$, and $***P < .001$.

is a well-established macrophage marker. Also *Ctsg*, a gene expressed mainly by neutrophils, showed a strong upregulation in mMCP6$^{-/-}$ cells. There was also a profound upregulation of *Mcpt8* (coding for mMCP8), an established marker of basophils. The upregulation of these non-MC lineage markers in tryptase-deficient cells was confirmed by qPCR analysis, whereas the expression of a control gene (*Cpa3*) was similar among the genotypes (Fig 6, *A*). Importantly, the upregulation of these genes was prominent in aged cell cultures, whereas approximately equal expression was seen in 6-week-old WT and mMCP6$^{-/-}$ cells (Fig 6, *B*). To further substantiate these findings, we performed flow cytometry analysis of the expression of *Emr1*/F4/80. In agreement with the gene array and qPCR data, profoundly higher cell surface expression of F4/80 was seen in aged mMCP6$^{-/-}$ versus WT cells (Fig 6, *C* and *D*). Elevated F4/80 surface expression was also seen in serglycin$^{-/-}$ cells (Fig 6, *C* and *D*). In contrast, surface expression of F4/80 was low and approximately equal among the genotypes in younger cells (7 weeks) (not shown).

Inhibition of histone acetyltransferase activity reverses the effects on gene expression seen in tryptase-deficient cells

To further address whether the aberrant gene expression seen in mMCP6$^{-/-}$ cells was due to increased histone acetylation, we assessed the effect of anacardic acid, a histone acetyltransferase inhibitor, on the expression of non-MC lineage markers (*Emr1, Mcpt8*). Indeed, incubation of mMCP6$^{-/-}$ cells with anacardic acid resulted in a significant decrease in the expression of *Emr1* and *Mcpt8* (Fig 6, *E*), hence supporting a causative link between histone acetylation and increased expression of non-MC lineage markers. Notably, whereas anacardic acid inhibited the expression of these genes in mMCP6$^{-/-}$ cells, no significant effect of anacardic acid was seen in WT cells (Fig 6, *E*). Moreover, anacardic acid had no significant effect on the expression of control genes whose expression were unaffected by the mMCP6 knockout (*Gapdh, Cpa3*) (Fig 6, *E*). We also assessed the effect of anacardic acid on the total levels of RNA in WT versus mMCP6$^{-/-}$ cells. As

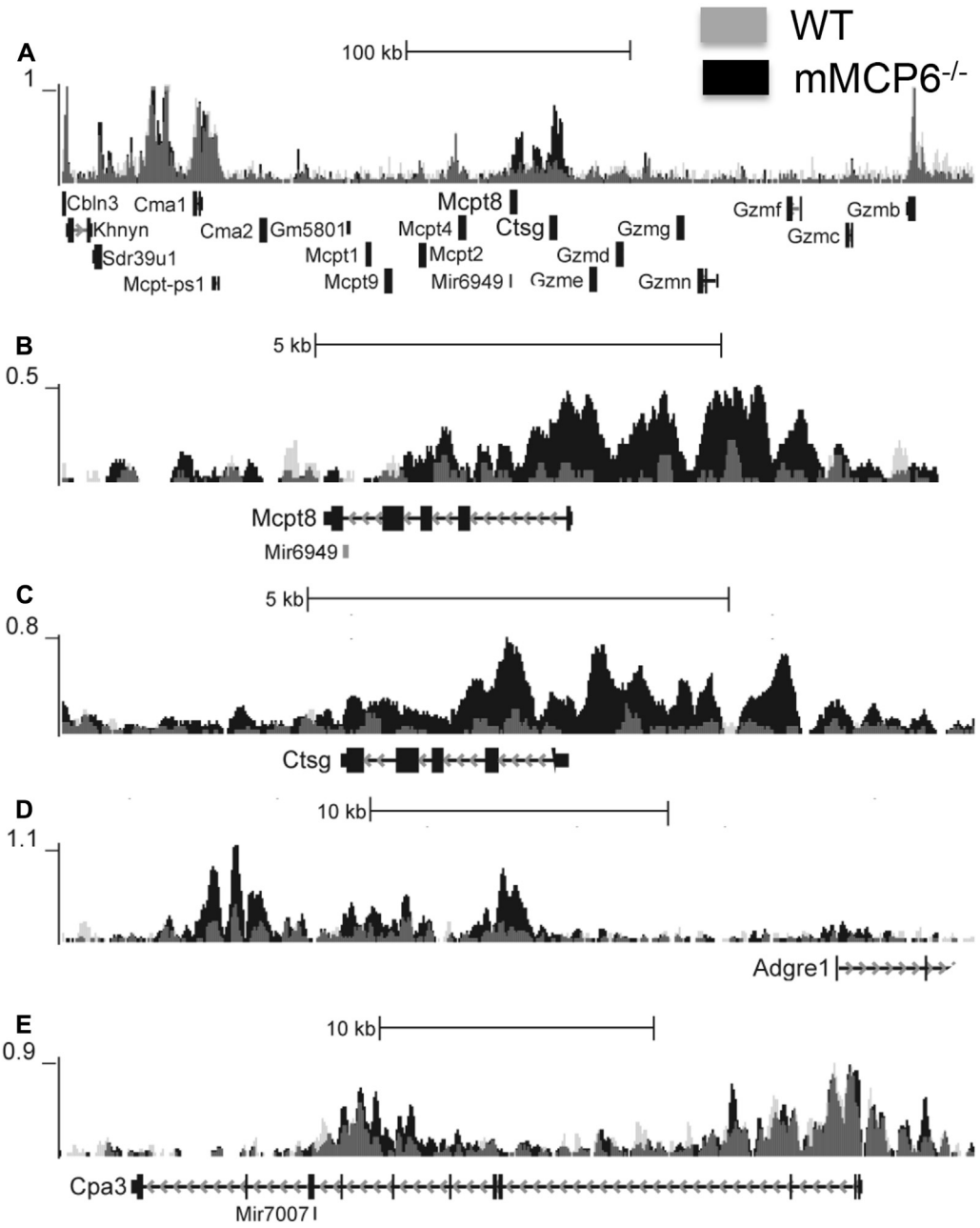


FIG 7. ChIP-seq enrichment for H2BK5ac. **A**, H2BK5ac signals in a 400 kb window of the chymase locus on chromosome 14. MACS wiggle tracks were normalized to reads per million. **B-E**, Signals around *Mcpt8, Ctsg, Emr1 (Adgre1)* and Cpa3 in WT (gray) and mMCP6$^{-/-}$ (black) cells.

seen in Fig 6, *F*, incubation of mMCP6$^{-/-}$ cells with anacardic acid produced a decrease in the amount of recovered RNA while not affecting cell viability (Fig 6, *G*). In contrast, histone acetyltransferase inhibition did not reduce the level of total RNA recovered from WT cells (Fig 6, *F*). Together, these data support that the upregulated expression of non-MC lineage genes in MCs lacking tryptase is an effect of increased histone acetylation, this in turn being a consequence of an accumulation of nonclipped core histones.

ChIP-seq analysis reveals an association between H2BK5ac and non-MC lineage genes

To substantiate the link between tryptase-deficiency and accumulation of H2BK5ac, we performed a genome-wide ChIP-seq analysis for H2BK5ac in mMCP6$^{-/-}$ cells and WT cells. We found on average a 2-fold higher enrichment for H2BK5ac in mMCP6$^{-/-}$ cells in enriched regions common between the 2 cell lines (see Fig E3 in this article's Online Repository at www.jacionline.org). Although this could indicate a genome-wide accumulation of H2BK5ac, we cannot rule out experimental variation in enrichment. Therefore, we normalized the signal in mMCP6$^{-/-}$ cells to that in WT to identify differentially enriched genes. As shown for the chymase locus on chromosome 14 in Fig 7, *A*, ChIP using the H2BK5ac antibody revealed similar enrichment around several genes in WT and mMCP6$^{-/-}$ cells. Strikingly, however, the enrichment of *Mcpt8* was much higher in mMCP6$^{-/-}$ than in WT cells, and the mMCP6$^{-/-}$ cells also showed a markedly higher enrichment of *Ctsg* than did WT

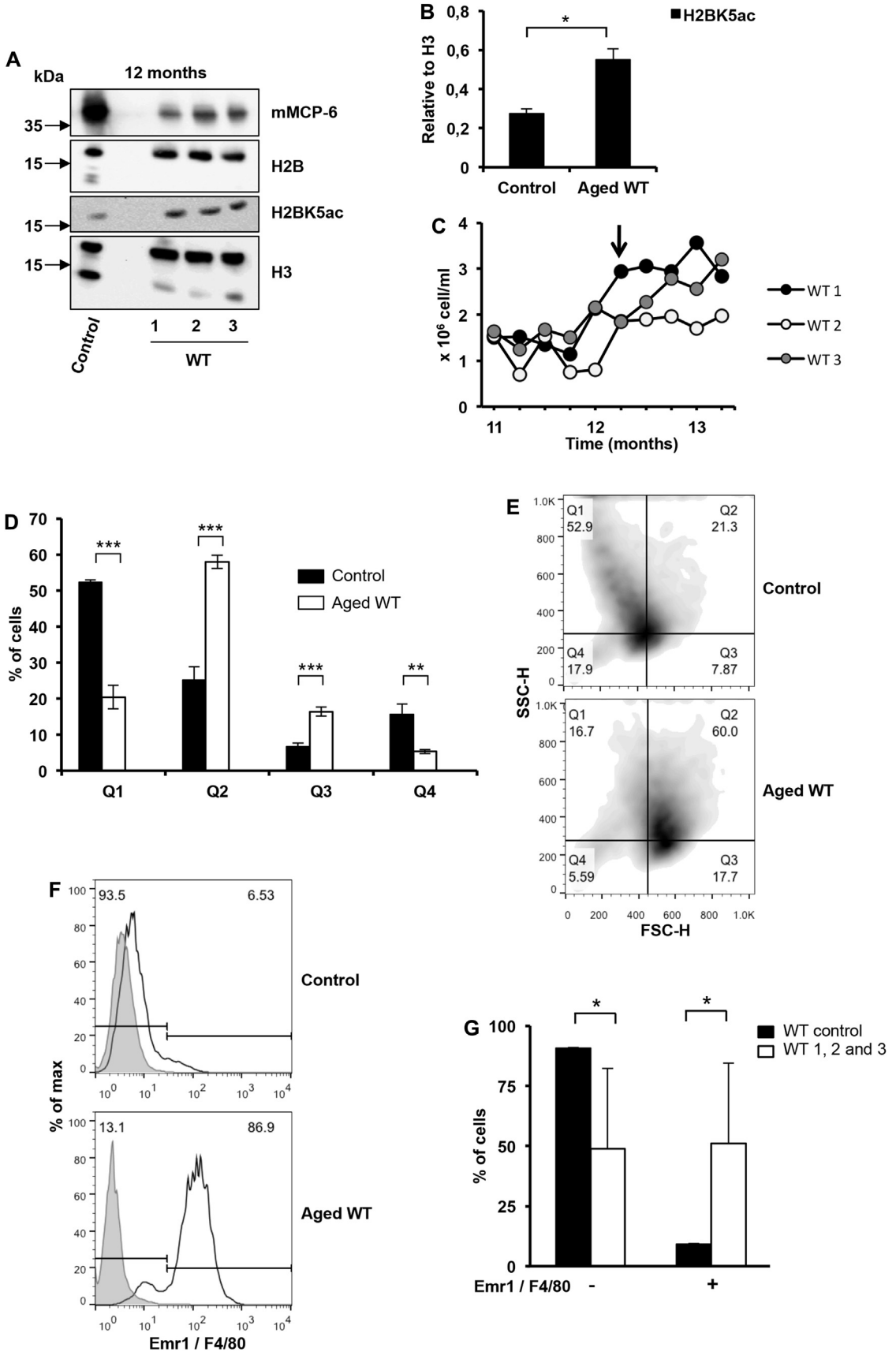


FIG 8. Reduced tryptase expression in aging WT MCs produces similar epigenetic events as in genetically tryptase-deficient cells. **A**, Western blot analysis of control (7-week-old) and aged (12-month-old) WT MCs

cells (Fig 7, *B* and *C*). Moreover, *Emr1 (Adgre1)* revealed a highly enriched region upstream of the transcription start site (Fig 7, *D*) whereas the control gene used in qPCR, *Cpa3*, had the same level of enrichment in both samples (Fig 7, *E*). Hence, these findings support a link between the accumulation of H2BK5ac and the gene expression patterns seen in the tryptase-deficient MCs. In a genome-wide comparison of the samples using read counts in larger windows (50 kb), we found that the *Mcpt8/Ctsg* region was among the 10 most upregulated regions (see Table E1 in this article's Online Repository at www.jacionline.org). Notably, a large number of genes in addition to *Mcpt8, Ctsg*, and *Emr1/Adgre1* showed accumulation of the H2BK5ac mark as a consequence of tryptase-deficiency (see Table E1and Fig E3).

Reduced tryptase expression in aging WT cells produces similar epigenetic events as in genetically tryptase-deficient cells

To further evaluate the age-dependent effects of tryptase on histone clipping, we followed cell cultures for further extended time periods, up to 12 months. Intriguingly, when culturing MCs for such time periods, we noted a partial loss of mMCP6 expression in WT cells (Fig 8, *A*). In agreement with this, we also noted a profound loss of mMCP6 expression *in vivo* in MCs of aging WT mice (Fig 2, *G*), whereas CPA3 and chymase expression was intact (Fig 2, *G* and Fig E2). In line with the effects of tryptase deficiency seen in genetically mMCP6-ablated cells, the partial loss of tryptase expression in aging WT cells was accompanied by lesser extents of H2B and H3 clipping (Fig 8, *A*) and by significant accumulation of the H2BK5ac mark (Fig 8, *A* and *B*). The loss of tryptase expression in aging WT cells was also accompanied by increased rates of proliferation (Fig 8, *C*), increased cellular size (Fig 8, *D* and *E*), and increased F4/80 surface expression (Fig 8, *F* and *G*), all of these findings being in line with the effects seen in genetically mMCP6-deficient cells (see Figs I, II, and VI, respectively). An increase in cellular size was also seen *in vivo,* as manifested in MCs of aging mice in which tryptase expression is downregulated (Fig 2, *G*). Hence, these data provide further support for a major role for tryptase in age-dependent regulation of epigenetic mechanisms through histone clipping.

DISCUSSION

The concept of histone clipping as an epigenetic mechanism in mammalian cells was introduced by Duncan et al[18] who showed that clipped H3 was present in differentiating embryonic stem cells and that silencing of cathepsin L resulted in attenuated H3 clipping. However, it is not clear whether such cathepsin L–mediated H3 clipping has a direct epigenetic effect as reflected by altered levels of H3 PTMs. Hence, although histone clipping as a concept has gained some attention,[19,20] there is still only limited data to support an epigenetic impact of this type of mechanism in mammalian cells. Moreover, it is notable that most previous studies on this topic have focused on the N-terminal clipping of H3 and C-terminal clipping of H2A,[20] whereas potential epigenetic effects of clipping of other core histones have not been studied.

In this study, we introduce clipping of H2B by tryptase as a novel epigenetic mechanism. The general consensus has been that tryptase has functions that are associated with its release to the extracellular milieu, while being biologically inert during intracellular storage.[4] Here we challenge this notion by showing that tryptase has a profound intracellular impact on MCs, by regulating its epigenome. Intriguingly, tryptase was found to be associated with chromatin and was shown to truncate both H3 and H2B of intact nucleosomes, thereby having the potential to abolish epigenetic modifications at these sites. Indeed, our data reveal that tryptase has an epigenetic regulatory role by preventing the accumulation of H2BK5 acetylation. Besides altering histone acetylation marks, we note that mammalian H2BK5 is adjacent to 2 potentially phosphorylatable residues, S4 and S6. Interestingly, phosphorylation at S14 in mammalian H2B and yeast H2B at S10 have been closely linked to apoptosis,[21,22] and in the case of *Saccharomyces cerevisiae* deacetylation of an adjacent K11 is required to initiate this apoptotic phosphorylation.[23] It remains unclear the extent to which, if at all, other PTMs besides H2BK5ac are impacted by the tryptase-mediated H2B cleavage that we have focused on in this study.

The present study suggests that cells lacking tryptase, with the consequent accumulation of H2BK5ac, undergo cell age–dependent extensive chromatin remodeling and other morphological changes, accompanied by loss of proliferative control. Moreover, there was a dramatic upregulation in tryptase null cells of genes that serve as markers of non-MC lineages. Importantly, the locus-specific accumulation of H2BK5ac at these genes was confirmed by ChIP-seq analysis, thus providing a causal link between the absence of tryptase and upregulated expression of non-MC lineage genes. These data hence introduce the possibility that tryptase-catalyzed histone clipping may serve as a mechanism to ensure preservation of cellular identity in aging cell populations, the absence of which will lead to aberrant expression of markers of other cell lineages. This raises the intriguing possibility that regulated acetylation of H2B could represent an epigenetic mechanism that regulates cell differentiation. In line with this notion, a recent study implicated H2BK5 acetylation in the regulation of epithelial-mesenchymal transition.[17] It is also striking that the observed aberrant accumulation of H2BK5ac is strongly correlated with the age of the cells. It is thus possible that loss of control over H2B acetylation could be among the epigenetic effects that may be associated with aging.[24]

taken from 3 independent cell cultures. Cells were analyzed for the levels of tryptase (mMCP6), H2B, H2BK5ac, and H3 as indicated. **B**, Quantification of H2BK5ac relative to H3 (n = 3). **C**, Quantification of cell numbers in the 3 independent WT MC cultures. The *arrow* indicates the time point for the Western blot analysis shown in (*A*). **D**, Quantification of the data in (*E*) (n = 3). **E**, Flow cytometry analysis showing density side/forward scatter plots of control and aged WT MCs. **F**, Flow cytometry analysis of the expression of Emr1/F4/80 in control and aged WT MCs. *Gray-shaded area*, isotype control. **G**, Percentage of Emr1/F4/80^{low} (−) and Emr1/F4/80^{high} (+) cells in control (7 weeks) and in 3 independent cultures (WT1, WT2, and WT3) of aged (12-month old) WT MCs (n = 3). Data are presented as mean values ± SEM; **P* < .05, ***P* < .01, and ****P* < .001.

The results presented here and in a previous study[5] indicate that tryptase has profound H3 and H2B clipping activity. However, whereas the epigenetic modification of H2B was affected in tryptase null cells, we did not see any effect of tryptase deficiency on the epigenetic signature of H3. Although we cannot at present with certainty explain this, it is notable that truncated H2B was found to be associated with chromatin, whereas we did not see chromatin-associated truncated H3. Hence, mMCP6-mediated H3 truncation may be a nonnucleosomal event, and it is therefore plausible that mMCP6 action on H3 does not affect its epigenetic signature. In contrast to H3, little is known concerning the biological impact of epigenetic H2B modifications. In most cases, histone acetylations are regarded as activating modifications. In agreement with such a notion, we see a dramatic increase in expression of numerous genes in cells exhibiting an enhanced H2BK5ac signal at their promoter proximal and distal regulatory elements, due to tryptase deletion. It was also evident that inhibition of histone acetylase activity has a corresponding inhibitory action on the expression of these genes, supporting a causative link between H2B acetylation and effects on gene expression.

An important question is whether tryptase-mediated H2B clipping has any biological implications beyond the MC context. Notably, although tryptase expression is largely MC-restricted, there is evidence that certain other cell types such as basophils and macrophages can express tryptase, albeit at lower levels.[25-28] Hence, it cannot be excluded that tryptase can have an epigenetic regulatory impact on non-MCs. Furthermore, it is conceivable that trypsin-like proteases distinct from tryptase, expressed by other cell lineages, may acquire similar histone-clipping activities as seen for MC tryptase.

Our data show that the epigenetic effects of tryptase deficiency largely overlap with those seen in cells lacking the proteoglycan serglycin. Because tryptase storage is strongly dependent on serglycin,[9] these findings reinforce the impact of tryptase on the epigenome of MCs. In contrast to tryptase, serglycin expression is relatively widespread.[29] Hence, serglycin might have epigenetic implications also when expressed by non-MC lineages, possibly by acting as a partner for histone-clipping proteases.

In summary, the present study introduces histone clipping by tryptase as a novel epigenetic mechanism, with implications for regulation of cell differentiation.

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Key messages

- **Tryptase is found in the chromatin of MCs, where it truncates N-terminal ends of histone 3 and histone 2B (H2B).**
- **The absence of tryptase leads to an age-dependent accumulation of the epigenetic mark H2BK5ac.**
- **The accumulation of H2BK5ac leads to extensive effects on the state of MC differentiation, including effects on gene expression, morphology, and proliferative control.**

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